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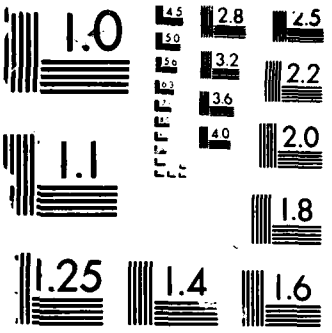

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To Whom It May Concern:

New England Medical Center Hospitals, Inc. submits original and multiple copies as requested of the above referenced annual report for our research project entitled "Regulation of Brain Neuropeptide Secretion by Lymphokines" under the direction of Dr. Seymour Reichlin of our Department of Medicine.

If you have any questions or need additional information, please call me.

Sincerely yours,

James E. Grayson, Director
Grants and Contracts

JEG:jl
Enclosure
cc: Dr. S. Reichlin

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(U) REGULATION OF BRAIN NEUROPEPTIDE SECRETION BY LYMPHOKINES

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Preliminary experiments outlined in the original application suggested that supernatants from mononuclear cell cultures stimulated with staphylococcal antigens or IL-2 could release SRIF from dispersed cortical neurons. Such activated supernatants are known to contain many cytokines, including interleukins-1 (IL-1). We elected to begin the study of this release activity by examining the effects of IL-1 and IL-2 in more detail, using pure products of recombinant DNA origin. Human recombinant interleukin-1-beta (rIL-1B) was tested in both cortical and diencephalic cells. Modest SRIF release activity was observed only at a concentration of 10^{-7} M.

To investigate the effect of chronic IL-1 exposure, cultures were incubated for 48 hours with various concentrations of rIL-1B and the cells and media assayed for SRIF.

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IL-1 was found to increase SRIF relative to control flasks in a dose dependant manner, but only at high concentrations.

Recombinant IL-2 was also tested in multiple experiments. No SRIF release activity was found in cortical cultures. Diencephalic cultures responded inconsistently, and then only to high concentrations of IL-2. To test the hypothesis that IL-1 might induce IL-2 responsiveness in neurons as it does in lymphocytes, diencephalic cells were incubated for 67 hours with 10^{-8}M rIL-1 and then tested with 10^{-8}M IL-2. Again, no effect was observed. Thus IL-2 appears to have little or no effect on SRIF release *in vitro*.

Returning to the crude supernatants, an IL-2 (1500U/mL) stimulated white cell supernatant (LAK) was serially (2 - 500X) and tested for SRIF release activity. No effects were seen. A cytokine-enriched concentrate obtained by passage of the supernatant over a sizing gel to select molecular weights less than 50,000 was likewise tested. Some dilutions of this preparation appeared to have activity. In both sets of experiments, however, the test supernatants contained added fetal calf serum. In the course of attempting to match the serum content of basal incubations to the serum content of the diluted supernatants, a confounding effect was discovered. Basal release of SRF was observed to increase in proportion to the amount of serum present.

To avoid this difficulty a supernatant IL-2 stimulated white cells incubated in serum-free medium was tested. No significant effect was observed. This lack of activity may have been due to a lack of lymphokine production by white cells cultured without serum. In any case, additional experiments with rigorously controlled serum content are needed to further characterize the supernatant release activity.

Corticotropin Releasing Factor and other Peptides.

Since IL-1 is known to stimulate ACTH release in whole animals. Dispersed diencephalic cells plated at 5 million cells per flask were assayed for the appearance of CRF in culture and the effects of IL-1 measured by RAI established for this purpose. Initial levles of 1000 pg/flask were found, which then declined until day 8, reaching a nadir of 2-300 pg/flask. Thereafter levels rose to a plateau of 600-800 pg/flask on days 10-30. Acute release experiments are underway. Initial studies of the chronic effects of IL-1 on cell content of CRF suggested that CRF is unchanged or decreased by IL-1, in contrast to the effect on SRIF. We have found that the use of increased amounts of glucose and serum can augment the culture content of CRF, but release experiments using such enriched cultures have not been yet done.

In order to study the acute effects of IL-1 on the hypothalamic release of CRF *in vitro*, a different system was employed. Whole hypothalami from young (150 g) male rats were rapidly dissected, placed into continuously gassed artificial CSF, and subjected to sequential incubations. In this study vasopressin was released by IL-1 as determined by RAI set up for this purpose. Our studies thus ahows a relatively peptide specific effect of IL-1 on vasopressin, but not on somatostatin secretion.

Diencephalic cultures were assayed for cell and media content of VIP and GRF. Both peptides were detectable, but not al levels that would permit lymphokine-stimulated release experiments at the current radioimmunoassay sensitivities. It may be more satisfactory to use whole hypothalamic incubates.

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